Maternal serum soluble fms-like tyrosine kinase-1—to—placental growth factor ratio distinguishes growth-restricted from non—growth-restricted small-for-gestational-age fetuses

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BACKGROUND: Fetal growth restriction secondary to chronic placental insufficiency is a major cause of perinatal morbidity and mortality. A significant proportion of fetuses with fetal growth restriction are small for gestational age, defined as a birthweight of ≤10th percentile. However, not all small-for-gestational-age fetuses are growth restricted. Some are constitutionally small and otherwise healthy. It is important to distinguish between small-for-gestational-age fetuses with and without fetal growth restriction to ensure appropriate interventions in small-for-gestational-age fetuses with fetal growth restriction and to minimize unnecessary interventions in healthy small-for-gestational-age fetuses. The maternal serum ratio of soluble fms-like tyrosine kinase-1 and placental growth factor is an indicator of placental insufficiency in the latter half of pregnancy. As such, the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio may be a clinically useful tool to distinguish between small-for-gestational-age fetuses with and without fetal growth restriction.

OBJECTIVE: This study aimed to determine whether the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio can distinguish between small-for-gestational-age fetuses with and without fetal growth restriction with a birthweight of ≤10th percentile.

STUDY DESIGN: A retrospective audit of 233 singleton pregnancies delivering an infant with a birthweight of ≤10th percentile corrected for gestational age with an antenatal maternal serum soluble fms-like tyrosine kinase-1—to—placental growth factor result was performed. Fetal growth restriction was defined as a birthweight of ≤10th percentile with an umbilical artery pulsatility index of >95th percentile, fetal middle cerebral artery pulsatility index of <5th percentile, amniotic fluid index of <6 cm, and/or cerebroplacental ratio of <1st percentile. The soluble fms-like tyrosine kinase-1—to—placental growth factor ratios before delivery between fetuses with and without fetal growth restriction (121 [fetal growth restriction] vs 112 [no fetal growth restriction]) were compared. The Student *t* test and Fisher exact test were used to compare cases and controls. The Mann-Whitney *U* test, linear regression analysis, and Spearman correlation coefficient (Rho) were used to examine associations between the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio and fetal outcomes to determine whether the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio served as a prognostic marker of fetal growth restriction severity.

RESULTS: The mean soluble fms-like tyrosine kinase-1—to—placental growth factor ratio was increased in fetal growth restriction cases compared with non—fetal growth restriction controls (234.3±25.0 vs 67.4±7.7, respectively; $P<.0001$). When controlling for preeclampsia, which is associated with placental insufficiency, fetal growth restriction cases still demonstrated an independent increase in the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio (effect size, 0.865; 95% confidence interval, 0.509—1.220; $P<.001$). The soluble fms-like tyrosine kinase-1—to—placental growth factor ratio was negatively correlated with birthweight percentiles in pregnancies delivering an infant with a birthweight of ≤10th percentile ($r=-0.3565$; $P<.0001$). This association was maintained for fetuses with fetal growth restriction ($r=-0.2309$; $P<.05$), whereas fetuses without fetal growth restriction had no significant correlation between the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio and neonatal birthweight percentiles.

CONCLUSION: The soluble fms-like tyrosine kinase-1—to—placental growth factor ratio was significantly higher in small-for-gestational-age fetuses with fetal growth restriction than small-for-gestational-age fetuses without fetal growth restriction, independent of preeclampsia. Further-

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more, the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio was negatively correlated with fetal growth restriction birthweight percentiles, suggesting that it may be a clinical measure of fetal growth restriction severity. Therefore, the ratio may usefully delineate fetal growth restriction from constitutionally small but otherwise healthy fetuses antenatally, allowing for timely interventions in small-for-gestational-age cases with fetal growth restriction and unnecessary interventions to be minimized in small-for-gestational-age cases without fetal growth restriction.

Key words: fetal growth restriction, placenta, small for gestational age, soluble fms-like tyrosine kinase-1—to—placental growth factor ratio, stillbirth

Introduction

Fetal growth restriction (FGR) describes the inability of a fetus to achieve its full biological growth potential during intrauterine life. It is a major cause of preventable stillbirths in high- and middle-income countries and is associated with a third of neonatal deaths in low-income areas.[1–3] Despite increased understanding of the pathogenesis underlying FGR, the antenatal detection and management of this condition remains a fundamental challenge for contemporary obstetrical practice.

Globally, the antenatal detection of FGR often relies on the assessment of maternal risk factors and routine symphysial fundal height (SFH) measurements. These screening tools are unreliable predictors of faltering growth[4] and fail to recognize 75% of pregnancies complicated by growth restriction antenatally.[5] Ultrasound (US) estimations of fetal weight and maternal and fetal Doppler abnormalities most commonly inform the current clinical management of suspected FGR.[6] However, universal third-trimester US screening for FGR was found to be a poor predictor of perinatal morbidity and mortality[7] and the significant cost burden associated with US further limits the breadth of its application to the wider pregnancy population in both high- and low-resource healthcare settings.[8] As such, poor antenatal detection of FGR has been associated with a significant increase in stillbirths, adverse perinatal outcomes, and adult-onset complications.[3,9,10]

A major cause of FGR is chronic placental insufficiency, which describes the inability of the placenta to supply sufficient oxygen and nutrients to the fetus and, thus, support fetal growth. This problem often appears late in pregnancy when fetal growth and placental metabolic demands increase significantly. Soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are placenta-derived maternal serum proteins, which are indicators of placental function.[11] Abnormalities in these markers may have the potential to recognize faltering fetal growth before the development of signs, such as decreased SFH in late pregnancy. Thus, the sFlt-1—to—PlGF ratio may facilitate the early detection and management of FGR cases and reduce the prevalence of associated adverse perinatal outcomes and perinatal deaths.

This study aimed to determine whether the value of the sFlt-1—to—PlGF ratio measured during the antenatal period can distinguish between small-for-gestational-age (SGA) fetuses with and without FGR (a birthweight [BW] of ≤10th percentile). The potential confounder preeclampsia (PE), which can be associated with FGR and chronic placental insufficiency, was controlled in a regression model. Furthermore, a correlation analysis between the sFlt-1—to—PlGF ratio and BW percentiles was undertaken to determine whether the ratio is eligible as a prognostic marker of FGR severity.

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Why was this study conducted?

This study aimed to determine whether the maternal serum soluble fms-like tyrosine kinase-1—to—placental growth factor (sFlt-1—to—PlGF) ratio can distinguish between small-for-gestational-age (SGA) fetuses with and without fetal grown restriction (FGR).

Key findings

The sFlt-1—to—PlGF ratio was significantly higher in SGA fetuses with FGR than in SGA fetuses without FGR, independent of preeclampsia.

What does this add to what is known?

The sFlt-1—to—PlGF ratio may be a clinically useful tool to distinguish between SGA fetuses with and without FGR with a birthweight of ≤10th percentile. This may avoid overintervention for constitutionally small and otherwise healthy fetuses and focus clinical resources on the surveillance and management of fetuses with FGR by placental insufficiency.

Materials and Methods

Study population

A retrospective cohort study of pregnancies at >20 0/7 weeks of gestation in whom an sFlt-1—to—PlGF ratio was undertaken was performed. sFlt-1—to—PlGF ratios were measured in patients who demonstrated signs and symptoms suspicious of PE without a preexisting diagnosis, as adopted from the prediction of short-term outcome in pregnant women with suspected preeclampsia study protocol inclusion criteria.[12] The setting was The Royal Women's Hospital, which is a tertiary maternity hospital in Melbourne, Australia. All sFlt-1—to—PlGF ratios collected between September 2016 and July 2019 were entered in a Research Electronic Data Capture database[13] with associated clinical information for subsequent audit evaluation. Ethics approval for audits based on information

in the sFlt-1 and PlGF database was granted by the institutional ethics committee (project approval number: AQA17/31).

The sFlt-1—to—PlGF ratios from singleton pregnancies who delivered an infant with a BW of ≤10th percentile were extracted from the database. Based on US findings of parameters associated with chronic placental insufficiency and poor fetal well-being, sFlt-1—to—PlGF ratio results were classified into cohorts with or without FGR. Data from multiple pregnancies and fetal cases of known congenital infections, malformations, or chromosomal abnormalities were excluded.

The maternal serum markers (sFlt-1, PlGF, and sFlt-1—to—PlGF ratio) were compared between cohorts with and without FGR. As serial maternal serum samples were taken in many cases, the final serum sample before delivery was selected on the basis of the assumption that this result would most accurately reflect relevant placental function.

TABLE 1
Parameters of poor fetal growth

Variable	Predictive factor	Diagnostic criteria
Primary indicators	AFI	AFI of <6 cm.
	UmA PI	Doppler PI of >95th percentile or absent or reversed end-diastolic flow.
	MCA PI	Doppler PI of <5th percentile.
	CPR	CPR ratio below 1.0.
Secondary indicators	CTG	Evidence of a nonreactive or nonreassuring trace.
	Apgar score	Measurements at 1 and 5 min were available. A score below 7 at 5 min was considered abnormal.
	NICU or SCN admission	NICU or SCN admissions indicated newborn compromise and need for medical support and monitoring.
	Placental histopathology	Histological features indicating uteroplacental insufficiency.

Table 1 outlines the primary and secondary assessments used to classify FGR and SGA cases in the current study.

AFI, amniotic fluid index; *CPR*, cerebroplacental ratio; *CTG*, cardiotocography; *MCA*, middle cerebral artery; *NICU*, neonatal intensive care unit; *PI*, pulsatility index; *SCN*, special care nursery; *UmA*, umbilical artery.

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Definitions

BW percentiles were assigned using the estimated fetal weight (EFW) of Hadlock et al's[14] 1991 growth formula.[15] This formula uses the gestational age (GA; weeks) and the EFW (grams) at US analysis to determine the fetal weight percentile. As BW percentiles are not routinely reported at delivery, the GA at delivery (weeks) and infant BW (grams) were substituted to estimate BW percentiles. BWs of ≤10th percentile were chosen for analysis, as this is the most widely recognized clinical surrogate of faltering growth and as most fetuses with FGR were likely to fall within these BW limits.[16]

FGR cases were defined as a BW of ≤10th percentile with umbilical artery pulsatility index (UmA PI) of >95th percentile, fetal middle cerebral artery pulsatility index (MCA PI) of <5th percentile, amniotic fluid index (AFI) of <6 cm and/or cerebroplacental ratio (CPR) of <1st percentile (Table 1). Fetuses without FGR were defined as a BW of ≤10th percentile in the absence of abnormalities in the aforementioned US and Doppler assessments.

PE was defined according to the American College of Obstetricians and Gynecologists guidelines as the onset of de novo hypertension (systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥ 90 mm Hg) commencing at or after 20 weeks of gestation with one or more signs of end-organ damage (eg, proteinuria, acute kidney injury, hepatic dysfunction, hemolysis, and FGR).[17] Eclampsia and hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) were classified as obstetrical emergencies associated with PE.

Serum immunoassays

Maternal serum samples of sFlt-1 and PlGF were collected by venipuncture in tubes without anticoagulant and were analyzed using the Roche Diagnostics Elecsys automated immunoassays and measured in picograms per milliliter. The sFlt-1 and PlGF concentrations and their ratio were available to treating clinicians during the antenatal period.

Statistical analysis

Continuous data were summarized using the mean and standard error of the mean (SEM) and assessed for nonrandom associations by applying the unpaired *t* test. Categorical data were summarized using the frequency and percentage and assessed for nonrandom associations by applying the Fisher exact test. Maternal serum analytes were evaluated using the Mann-Whitney *U* test, and data outcomes were expressed as mean and SEM. Normality was assessed using the Shapiro-Wilk test, where a *P* value of ≥.05 indicated a symmetrical numerical dataset.

To examine the trend of the sFlt-1—to—PlGF ratio relative to neonatal BW percentiles, Spearman rank correlation tests were performed and reported as Spearman rho (*r*). To control for potential confounding variables, multivariable linear regression models were performed. Logistic transformation of the outcome variable was required to ensure normal distribution. The outcomes were reported as effect size, standard error, and 95% confidence interval (CI). The 2-tailed *P* value of <.05 was considered statistically significant throughout.

Descriptive statistics and Spearman correlations were performed using

GraphPad Prism 8 software (GraphPad Software Inc, San Diego, CA). Linear regression models were performed using SPSS (SPSS Statistics for Macintosh; Version 27.0; IBM Corp, Armonk, NY).

Results

Study cohort and baseline characteristics

A total of 233 pregnancies were included in the current study, composed of 121 fetuses with FGR and 112 fetuses without FGR. Maternal characteristics, birth outcomes, and fetal assessments of well-being are shown in Table 2.

Antenatal baseline characteristics revealed no discernable difference between the groups other than the

TABLE 2
Baseline maternal and fetal characteristics of the study population

Baseline characteristic	Classification	BW of ≤10th percentile		
		No FGR (n=112)	FGR (n=121)	*P* value
Baseline maternal characteristics				
Maternal age, mean (SEM)		35.460 (0.5396)	35.018 (0.5012)	.5486
BMI, mean (SEM)	Height (cm)	161.072 (1.123)	161.103 (1.270)	.9856
	Prepregnancy weight (kg)	70.869 (1.858)	69.530 (1.662)	.5915
	Prepregnancy BMI (kg/m^2)	26.867 (0.636)	26.588 (0.603)	.7507
Maternal risk factors, n (%)	Preeclampsia, Eclampsia or HELLP syndrome	42 (37.50)	82 (67.77)	<.0001
	Gestational diabetes mellitus	19 (16.96)	15 (12.40)	.3569
Parity, n (%)	0	76 (67.86)	72 (59.50)	.2205
	1	20 (17.86)	28 (23.14)	.3356
	>1	16 (14.29)	21 (17.36)	.5921
Pathology, mean (SEM)	Gestation at sampling (wk)	35.74 (0.3454)	33.48 (0.3672)	<.0001
Onset of delivery, n (%)	Induction of labor	58 (51.79)	44 (36.36)	.0245 (*)
	No labor	34 (30.36)	71 (58.68)	<.0001 (****)
	Spontaneous labor	20 (17.86)	6 (4.96)	<.01(**)
Mode of delivery, n (%)	Normal vaginal delivery	37 (33.04)	24 (19.83)	<.05 (*)
	Instrumental delivery	28 (25.00)	7 (5.79)	<.0001 (****)
	Emergency cesarean delivery	4 (3.57)	5 (4.13)	>.9999
	Elective cesarean delivery	43 (38.39)	85 (70.25)	<.0001 (****)
Baseline fetal characteristics				
Assessment, mean (SEM)	Gestational age at US (wk)	34.84 (0.39)	32.88 (0.38)	.0004 (***)
Bedside test, nonreactive, n (%)	Cardiotocography	1 (0.89)	10 (8.26)	.0105 (*)
Primary assessments, mean (SEM)	Amniotic fluid index (cm)	12.46 (0.3985)	11.73 (0.4127)	.2155
	Umbilical artery PI	1.005 (0.02712)	1.223 (0.03763)	<.0001 (****)
	Middle cerebral artery PI	1.840 (0.05432)	1.676 (0.04245)	.0184
	Cerebroplacental ratio	1.859 (0.06062)	1.476 (0.06114)	<.0001 (****)
Fetal outcomes, mean (SEM)	BW (g)	2554 (49.16)	1829 (57.10)	<.0001 (****)
	Gestation at delivery (wk)	38.07 (0.2944)	34.78 (0.3484)	<.0001 (****)
	BW percentile	5.982 (0.2582)	2.822 (0.2439)	<.0001 (****)
	Apgar score at 1 min	7.946 (0.1738)	7.083 (0.2390)	.0043
	Apgar score at 5 min	8.696 (0.08587)	8.331 (0.1702)	.0623

HELLP, hemolysis, elevated liver enzymes, and low platelet count; *PI*, pulsatility index; *SEM*, standard error of the mean; *US*, ultrasound.

p-value is less than 0.05, it is flagged with one star (*); If a p-value is less than 0.01, it is flagged with 2 stars (**); If a p-value is less than 0.001, it is flagged with three stars (***); If p-value is less than 0.0001 it is flagged with four stars (****).

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incidence of PE and the GA at serum sampling (Table 2). Cases with fetuses with FGR were significantly more likely to be associated with PE (67.8% [FGR] vs 37.5% [no FGR]; $P<.0001$) and underwent sFlt-1 and PlGF sampling 2 weeks and 2 days earlier than the population without FGR within the study period (33.5 weeks [FGR] vs 35.7 weeks [no FGR]; $P<.0001$) on average.

At delivery, compared with fetuses without FGR, a smaller proportion of fetuses with FGR had spontaneous onset of delivery (17.9% vs 5.0%, respectively; $P<.01$) and normal vaginal deliveries (33.0% vs 19.8%, respectively; $P<.05$) and a higher proportion experienced no labor (30.4% vs 58.7%, respectively; $P<.0001$) and elective cesarean deliveries (38.4% vs 70.3%, respectively; $P<.0001$). Fetuses with FGR were 725 g and 3.2 percentiles smaller ($P<.0001$) and were born 3 weeks and 2 days earlier ($P<.0001$) on average.

Using serum soluble fms-like tyrosine kindase-1 and placental growth factor to distinguish between fetuses with and without fetal growth restriction

Maternal serum analyte concentrations were compared between fetuses with and without FGR (Table 3, Figure 1). Fetuses with FGR had a higher mean sFlt-1 level ($P<.0001$) and lower mean PlGF level ($P<.0001$) than fetuses without FGR. Correspondingly, the mean sFlt-1−to−PlGF ratio was significantly higher in fetuses with FGR (mean±SEM: 234.3±25.0 vs 67.4±7.7 respectively; $P<.0001$).

In pregnancies that developed PE, HELLP, or eclampsia during the antenatal or postnatal period, fetuses with FGR had a higher mean sFlt-1 level ($P<.001$), lower mean PlGF level ($P<.0001$), and higher mean sFlt-1−to−PlGF ratio ($P<.0001$) than fetuses without FGR. Significant variations of sFlt-1, PlGF, or the sFlt-1−to−PlGF ratio were not observed between FGR and non-FGR cases in pregnancies that did not develop PE.

Controlling for potential confounders of the soluble fms-like tyrosine kinase-1−to−placental growth factor ratio

A multivariable log-linear regression model assessed the association between FGR and the sFlt-1−to−PlGF ratio while controlling for PE as a confounding variable.

Confounders of the sFlt-1−to−PlGF ratio were assumed on the basis of antenatal variations of maternal baseline characteristics (Table 2). PE and GA at sampling were included in the initial saturated regression model (Supplementary Table 1). This model revealed that GA did not have a statistically significant association with the sFlt-1−to−PlGF ratio ($P=.482$). As such, the final model controlled for PE alone (Table 4).

When controlling for PE, sFlt-1 (ß=0.424; 95% CI, 0.240−0.608; $P<.001$) and the sFlt-1−to−PlGF ratio (ß=0.865; 95% CI, 0.509−1.220; $P<.001$) were positively correlated and independently associated with an FGR outcome. Conversely, PlGF (ß=−0.471; 95% CI, −0.704 to −0.239; $P<.001$) was negatively correlated and independently associated with an FGR outcome.

Correlation of birthweight percentiles with the soluble fms-like tyrosine kinase-1−to−placental growth factor ratio

The sFlt-1−to−PlGF ratio concerning neonatal BW percentiles was evaluated within cohorts with and without FGR (Figure 2). The sFlt-1−to−PlGF ratio was negatively correlated with the total population of BWs of ≤10th percentile ($r=-0.3565$; $P<.0001$). This association was maintained when assessing fetuses with FGR independently ($r=-0.2309$; $P<.05$). Fetuses without FGR had no statistically significant correlation with the sFlt-1−to−PlGF ratio.

TABLE 3
sFlt-1, PlGF, and sFlt-1−to−PlGF ratio compared between fetuses with and without FGR

Maternal serum analytes	Birthweight of ≤10th percentile		
	No FGR (n=112)	FGR (n=121)	*P* value
sFlt-1 (pg/mL)	5597.00±387.20	10,223.00±643.10	<.0001
PlGF (pg/mL)	221.70±28.03	110.10±11.92	<.0001
sFlt-1−to−PlGF ratio	67.41±7.71	234.30±25.01	<.0001

Maternal serum analytes	Preeclampsia, HELLP, or eclampsia		
	No FGR (n=42)	FGR (n=82)	*P* value
sFlt-1 (pg/mL)	7800.00±803.80	11,827.00±784.80	<.001
PlGF (pg/mL)	151.90±24.58	76.61±9.19	<.0001
PlGF (pg/mL)	151.90±24.58	76.61±9.19	<.0001

Maternal serum analytes	No preeclampsia, HELLP, or eclampsia		
	No FGR (n=70)	FGR (n=39)	*P* value
sFlt-1 (pg/mL)	4275.19±296.70	6849.23±921.90	.068053 (ns)
PlGF (pg/mL)	263.60±41.71	180.50±28.63	.2571 (ns)
sFlt-1−to−PlGF ratio	42.46±4.72	121.82±37.89	.0645 (ns)

Data are presented as mean±standard error of the mean, unless otherwise indicated.

FGR, fetal grown restriction; *HELLP*, hemolysis, elevated liver enzymes, and low platelet count; ns, not statistically significant; *PlGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1.

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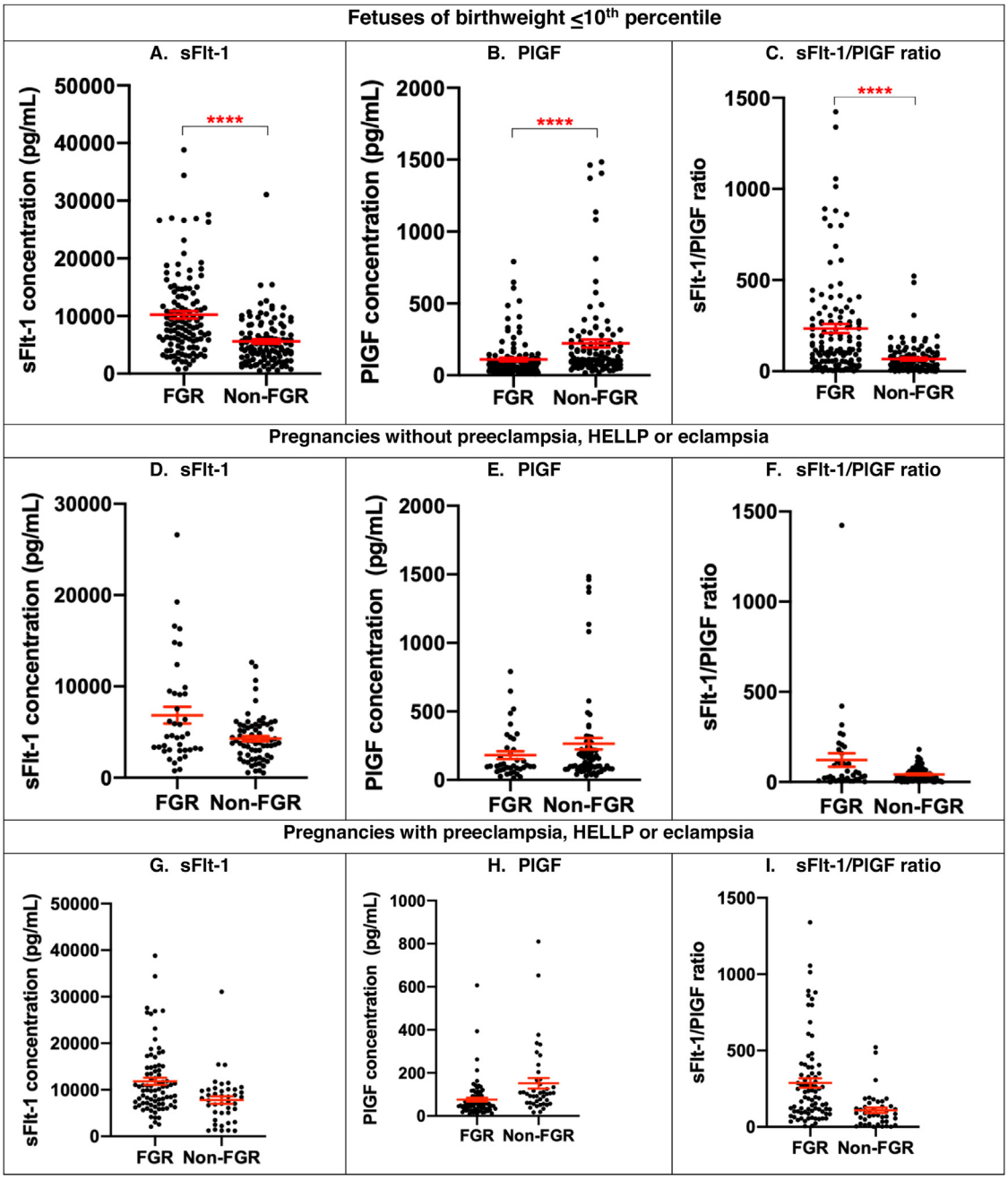
FIGURE 1
Maternal serum analytes compared between fetuses with and without FGR



FGR, fetal growth restriction; *PlGF*, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1.

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Comment

Principal findings

The principal finding of this study was that the maternal serum sFlt-1—to—PlGF ratio is increased in fetuses with FGR compared with fetuses without FGR with a BW of ≤10th percentile. This relationship remained significant when controlling for PE, which is also a disease associated with placental dysfunction and elevated sFlt-1—to—PlGF ratios.[18] Furthermore, the sFlt-1—to—PlGF ratio and neonatal BW percentiles were negatively correlated in fetuses with FGR.

The outcomes of this study suggest that an increased sFlt-1—to—PlGF ratio may distinguish between fetuses with FGR secondary to chronic placental insufficiency and constitutionally small but otherwise healthy fetuses without FGR with a BW of ≤10th percentile. This finding facilitates improved antenatal detection of FGR and may further guide appropriate antenatal interventions in SGA fetuses.

TABLE 4
Linear regression model assessing the association among FGR, PE, and the maternal serum markers

Predictive variable	ß	SE	Lower 95% CI	Upper 95% CI	*P* value
sFlt-1					
Intercept	8.148	0.073	8.004	8.292	<.001
FGR	0.424	0.093	0.240	0.608	<.001
PE	0.625	0.094	0.440	0.809	<.001
PlGF					
Intercept	5.139	0.092	4.957	5.321	<.001
FGR	−0.471	0.118	−0.704	−0.239	<.001
PE	−0.591	0.118	−0.824	−0.358	<.001
sFlt-1−to−PlGF ratio					
Intercept	3.054	0.142	2.774	3.333	<.001
FGR	0.865	0.181	0.509	1.220	<.001
PE	1.192	0.181	0.836	1.548	<.001

Table 4 displays the multivariate log-linear regression model demonstrating the association between FGR and the maternal serum analytes when adjusting for PE as a confounder. Effect size estimates (ß), SE, 95% CI, and the associated *P* values are outlined in the table. A *P* value of <.05 is considered a statistically significant result.

CI, confidence interval; *FGR*, fetal growth restriction; *PE*, preeclampsia; *PlGF*, placental growth factor; *SE*, standard error; *sFlt-1*, soluble fms-like tyrosine kinase 1.

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The use of the soluble fms-like tyrosine kinase-1−to−placental growth factor ratio in distinguishing fetal growth-restricted and non−fetal growth-restricted fetuses

The findings of the current study are novel in their delineation of FGR vs constitutionally small fetuses within an SGA cohort. Several studies have compared SGA cohorts with average-for-gestational-age (AGA) fetuses with a BW of ≥10th percentile.[19−21] Clinically, this distinction may be useful to predict reduced BW in the wider pregnancy population and delineate a high-risk cohort requiring additional surveillance. However, as a large proportion of SGA fetuses have normal perinatal outcomes,[22] this distinction fails to identify true fetuses with FGR and may encourage unnecessary antenatal intervention. Studies adopting a more accurate definition of FGR have demonstrated a higher sFlt-1−to−PlGF ratio in fetuses with FGR with poor perinatal outcomes, such as preterm delivery, lower neonatal BW, and perinatal mortality.[23−25] However, these studies do not directly distinguish fetuses with and without FGR.

The outcomes of this study are broadly consistent with the fetal longitudinal assessment of growth study,[19] which found an increased sFlt-1−to−PlGF ratio in fetuses with a BW of <10th percentile (SGA) compared with fetuses with a BW of ≥10th percentile (AGA), sampled at 36 weeks of gestation. Several studies demonstrated this association when comparing SGA fetuses with AGA cohorts, which were presumed to be physiologically well.[20,21] Few studies have adopted a more rigorous definition for FGR, including a combination of EFW below the 10th percentile, reduced AFI, and increased UmA PI.[26−29] In all instances, the sFlt-1−to−PlGF ratio was statistically significantly higher in fetuses with FGR than AGA and SGA controls.[26−29]

Controlling for potential confounders of the soluble fms-like tyrosine kinase-1−to−placental growth factor ratio

Given that PE is associated with an elevated sFlt-1−to−PlGF ratio,[18,30,31] a multivariable log-linear regression analysis was undertaken to determine whether FGR is independently associated with the sFlt-1−to−PlGF ratio in the presence and absence of PE. The regression analysis demonstrated that the increased sFlt-1−to−PlGF ratio in FGR cases was independent of PE.

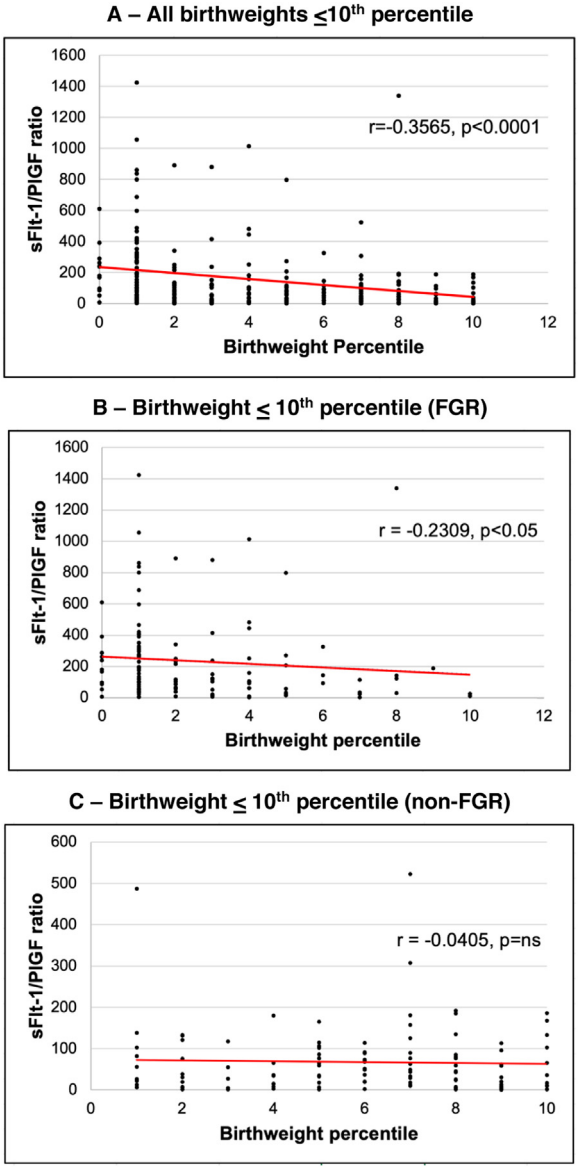
The outcomes of this study are consistent with previous studies that have excluded PE from their study population to explore the independent association between FGR and the sFlt-1−to−PlGF ratio. However, it is important to note that the existing literature compares true FGR or SGA fetuses to AGA cohorts, where reduced fetal weight or BW may be a potential confounder. Triunfo et al[32] compared 80 SGA and 80 AGA fetuses in the third trimester of pregnancy and found that the sFlt-1−to−PlGF ratio was significantly increased in SGA fetuses in the absence of early-onset PE. Similar outcomes have been reported in the second[33] and third trimesters of pregnancy.[19,32,34] Studies adopting a robust definition of FGR beyond reduced fetal weight or BW have reported statistically significant elevations of the sFlt-1−to−PlGF ratio compared with AGA cohorts in the absence of PE.[26,35]

Correlation of the soluble fms-like tyrosine kinase-1−to−placental growth factor ratio with neonatal birthweight outcomes

Low BW percentiles do not necessarily imply pregnancy pathology. However, it indicates an increased risk of adverse perinatal outcomes.[36] As BW percentiles decrease, the proportion of fetuses with FGR substantially increases.[37] The findings of the current study are consistent with Kwiatkowski et al,[38] who found an inverse correlation between BW (grams) with the sFlt-1−to−PlGF ratio in fetuses with a BW of <10th percentile (r=−0.46; P<.001) and Dathan-Stumpf et al,[39] who found a similar inverse correlation in singleton pregnancies (r=−0.58; P<.001).

When delineating SGA fetuses, the inverse correlation is evident in FGR cases and not evident in non-FGR cases. This outcome was expected, as fetuses without FGR represent a constitutionally small population unassociated

FIGURE 2
Correlation between birthweight percentiles and the sFlt-1—to—PlGF ratio



PlGF, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1.

Rajiv. Soluble fms-like tyrosine kinase-1—to—placental growth factor ratio and fetal growth restriction. Am J Obstet Gynecol Glob Rep 2024.

with placental insufficiency and, therefore, unlikely to demonstrate an association with the sFlt-1—to—PlGF ratio.

Clinical implications

Our findings suggest a role for the sFlt-1—to—PlGF ratio to more accurately distinguish FGR cases secondary to chronic placental insufficiency from constitutionally small fetuses without FGR in the SGA population. The negative correlation between the sFlt-1—to—PlGF ratio and neonatal BW outcomes further suggests that the value of the sFlt-1—to—PlGF ratio may predict the severity of adverse perinatal outcomes in fetuses with FGR. This may facilitate clinical decisions to escalate surveillance, hospitalize high-risk pregnancies, and influence the timing of delivery. This is particularly relevant in late pregnancy FGR diagnosed after 34 weeks of gestation, which are more likely to suddenly deteriorate in the absence of clinical signs detected by antenatal US surveillance.[40,41]

Research implications

Multivariate analysis studies have indicated that the sFlt-1—to—PlGF ratio used in conjunction with US analysis is a better predictor of poor perinatal outcomes than the use of US analysis alone.[23,42] However, validated ratio cutoffs to guide clinical intervention are yet to be established for the sFlt-1—to—PlGF ratio in cohorts with FGR. Such research would allow for the development of management algorithms in fetuses with FGR.

Further prospective trials with robust definitions of FGR and larger study populations are required before the implementation of the sFlt-1—to—PlGF ratio as a routine screening tool in antenatal care.

Strengths and limitations

This study has characterized the role of sFlt-1—to—PlGF ratio in distinguishing fetuses with FGR from constitutionally small fetuses. Previous studies have compared SGA fetuses with FGR to AGA cohorts, which mimics the screening outcomes achieved by existing tools, such as SFH assessments to define a high-risk cohort based on reduced fetal size alone. The outcomes of the current study suggest the potential for the sFlt-1—to—PlGF ratio to further delineate fetuses with and without FGR within an SGA cohort.

A strength of this study was the robust definition of FGR and the relatively large sample of fetuses with FGR. By comparison, previous studies evaluating cohorts with FGR have included fewer than 30 pregnancies.[21,26,29] This limits the statistical power of the outcomes and the generalizability of their findings. Limited sample size is often an inherent compromise of prospective studies in this field as FGR is an uncommon outcome.[43] The retrospective nature of the current study allowed for the inclusion of 3 years of data and 121 FGR cases to address the limitations of the existing literature.

Another strength of this study was to control for PE as a variable that can influence sFlt-1−to−PlGF ratio results using a regression analysis. Given that PE may occur in conjunction with cases of suspected FGR,[44,45] the interpretation of the sFlt-1−to−PlGF ratio in the presence and absence of PE revealed the independent association between FGR and the sFlt-1−to−PlGF ratio.

Given the retrospective study design, the strength of the recommendations provided in this study will need to be further validated in prospective studies. Furthermore, clinicians were unblinded to the sFlt-1−to−PlGF ratio outcomes during the antenatal period. This may have introduced performance bias toward the management and subsequent postnatal outcomes of the fetuses with and without FGR, as clinicians at this health service were aware of the association between the sFlt-1−to−PlGF ratio and placental dysfunction in the context of PE. However, this limitation was unavoidable because of the retrospective nature of the data analyzed.

Finally, maternal serum sampling was not limited to a specific gestational period of pregnancy; however, GA at sampling revealed no confounding effect during the initial regression analysis performed in this study.

Conclusions

Our study suggests that the sFlt-1−to−PlGF ratio is a useful clinical tool to more accurately delineate high-risk SGA fetuses. This may improve the escalation of pregnancy surveillance, improve the estimation of the timing of delivery, encourage early implementation of appropriate postnatal care, and reduce the incidence of preventable stillbirths in this population.

Glossary

AGA: Average for gestational age
AFI: Amniotic fluid index
BMI: Body mass index
BW: Birthweight
CPR: Cerebroplacental ratio
CTG: Cardiotocography
EFW: Estimated fetal weight
FGR: Fetal growth restriction
GA: Gestational age
HELLP: Hemolysis, elevated liver enzymes, and low platelet count
MCA: Middle cerebral artery
NICU: Neonatal intensive care unit
PE: Preeclampsia
PlGF: Placental growth factor
PI: Pulsatility index
SEM: Standard error of the mean
SCN: Special care nursery sFlt-1: Soluble fms-like tyrosine kinase-1
SFH: Symphysial fundal height
SGA: Small for gestational age
UmA: Umbilical artery
US: Ultrasound

CRediT authorship contribution statement

Prithi Rajiv: Data curation, Formal analysis, Writing − original draft, Writing − review & editing. **Thomas Cade:** Formal analysis. **Jennifer Dean:** Data curation. **Gabriel Davis Jones:** Data curation, Formal analysis, Software. **Shaun P. Brennecke:** Conceptualization, Resources, Supervision, Visualization, Writing − review & editing.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xagr.2023.100302.

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